

Gram +VE Bacteria

Genus : Clostridium



أ.د / جمال يونس

genus: Clostridium

general characters:

I Morphology:

- ◆ stain used → Gram's stain
 - ◆ staining reaction → Gram +ve
 - ◆ shape → straight rods except *C. spiroforme* which is curved or spiral cells
 - ◆ size → medium to large
 - ◆ arrangement → single, pairs or in short chains except:
 - 1- *C. septicum* → produce long filaments (snake-like) in films prepared from peritoneal surface of liver.
 - 2- *C. novyi* → produce long filaments
 - ◆ Motility → Motile with peritrichous flagella except *C. tetani* and *C. perfringens* (non-motile)
 - ◆ Capsule → Non-capsulated except *C. perfringens*
 - ◆ form endospores:
 - 1- size → larger in diameter than that of vegetative cells causing bulging of cell.
 - 2- shape → round or oval
 - 3- position → central, subterminal or terminal
- produce a characteristic diagnostic morphological feature:
- ① Round, terminal (drum stick appearance) → *C. tetani*
 - ② oval, subterminal (spoon like shape) → *C. novyi* - *C. botulinum*
 - ③ oval, central or subterminal → *C. perfringens* (rarely sporulated)
 - ④ oval, central or subterminal (lemon shape) → *C. septicum* and *C. chauvoei*.

II Isolation:

- ◆ O₂ req. → obligatory anaerobic due to lack of resp. enzymes (catalase, oxidase and peroxidase)
- ◆ opt. temp → 37 °C
- ◆ opt. pH → Neutral
- ◆ Incubation time → 1-3 days
- ◆ Media used for their growth and cultivation:
 - ① Enriched liquid media → cooked meat broth (Robertson's medium), Liver-Liver broth (Tarozzi medium)
 - ② Solid media → sheep or horse blood agar, reinforced clostridial agar.

III products:

They produce exotoxins.

Classification:

- ① acc. to biochemical activities on proteins and CHO in cooked meat medium:
 - 1- proteolytic group:
decompose protein and turns meat particles into black colour with foul odour → e.g. *C. histolyticum*.
 - 2- saccharolytic (gas gangrene) group:
ferment CHO in meat → pink colour with production of large amount of gases (stormy fermentation) e.g. *C. perfringens*, *C. septicum*, *C. novyi* and *C. chauvoei*.
 - 3- proteolytic and saccharolytic group:
e.g. *C. botulinum*.
 - 4- Non-proteolytic and non-saccharolytic group: *C. tetani*

② acc. to pathogenicity:

Saprophytic Clostridia

- Commonly Found in Soil, Sewage and Water in Sporulated Form
- However, pathogenic clostridia (such as *C. tetani*, *C. septicum*, *C. chauvoei* and *C. novyi*) Found in Soil as saprophytes

pathogenic Clostridia

- Normal inhabitant in the intestinal tract of man and animals and produce the disease under certain conditions.
- divided into 2 groups acc. to mechanism of disease production:

Invasive (gas gangrene) group

- Invade and multiply in internal organs with production of Large amount of Less potent toxins.

Such as:

C. perfringens
C. septicum
C. chauvoei
C. novyi

Non-Invasive (highly toxic) group

- have no power to invade living tissues
- their pathogenicity depend on production of highly powerful neurotoxins either in:

- ① Localized infected deep wound (*C. tetani*)
- ② Contaminated canned or salted fish (*C. botulinum*)

③ acc. to position of Spore and gelatin

Liquefaction:

1- Subterminal spores with gelatin hydrolysis group:

C. botulinum, *C. perfringens*, *C. chauvoei*, *C. septicum* and *C. novyi*

2- Subterminal spores without gelatin hydrolysis group:

include Saprophytic clostridia e.g. *C. butyricum*

3- Terminal spores with gelatin hydrolysis group:

C. tetani

4- Terminal spores without gelatin hydrolysis group:

include Saprophytic clostridia e.g. *C. tertium*

Toxin production

CL. tetani

→ 2 types of exotoxins are produced by the vegetative form and not by spores.

	Tetanolysin (Haemolysin)	Tetanospasm (Neurotoxin)
① action	Haemolysis of RBCs	acts on nervous system causing contractions of voluntary muscles due to ↑ muscular hyperactivity.
② Lethal effect to mice	—	+
③ effect of: a- Heat b- oxygen	Labile Labile	Labile stable
④ producer strains and antigenic structure	all toxigenic and non-toxigenic strains e.g. C. tetanoides and C. tetanomorphum	all toxigenic serotypes. It has one antigenic structure i.e. it is neutralized by antitoxin of all serotypes

Tetanospasm

- chemical structure → simple protein
- effect of:
 - a- acid → Labile
 - b- 0.3% Formalin → Labile and converted into toxoid (important for vaccine preparation)
- It binds irreversibly to gangliosides of nerve cells so, antitoxin is not effective when binding occurs.

CL. botulinum

produce 8 antigenically distinct exotoxins (antitoxin of one type not neutralize the toxins of other types)

Type	A	B	C _α	C _β	D	E	F	G
toxin	A	B	C ₁ (C ₂)	C ₂ , D (C ₁)	C ₂ , D	E	F	G

● They are the most powerful toxins in the world.

● Effect of:

a- Heat → Heat stable at 60°C/30 min.
→ Heat Labile at 80°C/30-40 min.

b- acid → acid-stable (Not affected by gastric juice)

● Mode of action:

interfere with the mechanism of release of acetyl choline from the motor nerve ending of parasympathetic nervous system causing paralysis.

● Neurotoxins → absorbed through digestive mucosa → symptoms appear after 36-96 hrs and death occurs due to paralysis of respiratory center.

CL. perfringens (C. Welchii)	C. Septicum	C. chauvoei	C. novyi								
<p>pathogenic strains of C. perfringens produce <u>12 toxic (enzymatic) factors</u> which are differentiated into 2 groups:</p> <p>① <u>Major toxins</u>: 4 types</p> <ul style="list-style-type: none"> ● <u>α-toxin</u> → has Lecithinase activity. ● <u>B-toxin</u> → trypsin-Labile ● <u>E and iota</u> → protoxins which need proteolytic enzymes (such as pepsin or trypsin) to be activated. <p><u>CL. perfringens</u>:</p> <p>→ Type A → α</p> <p>→ Type B → α, B and E</p> <p>→ Type C → α and B</p> <p>→ Type D → α and E</p> <p>→ Type E → α and iota</p> <p>② <u>Minor toxins</u>: 8 types</p> <table border="0"> <tr> <td>γ (Gamma)</td> <td>μ (Mu)</td> </tr> <tr> <td>δ (Delta)</td> <td>η (eta)</td> </tr> <tr> <td>θ (Theta)</td> <td>κ (Kappa)</td> </tr> <tr> <td>ν (nu)</td> <td>λ (Lambda)</td> </tr> </table>	γ (Gamma)	μ (Mu)	δ (Delta)	η (eta)	θ (Theta)	κ (Kappa)	ν (nu)	λ (Lambda)	<p>produce <u>8 types of toxins</u>:</p> <ol style="list-style-type: none"> ① α-toxin (Lethal and necrotizing) ② haemolysin ③ haemagglutinin ④ Fibrinolysin ⑤ desoxyribonuclease ⑥ hyaluronidase ⑦ Collagenase ⑧ Neuraminidase <p>→ α-toxin and haemolysin are shared antigenically with that of C. chauvoei</p> <p>● antisera of C. septicum neutralize the pathogenicity of C. septicum as well as C. chauvoei.</p> <p>● antisera of C. chauvoei neutralize the pathogenicity of C. chauvoei only.</p>	<p>produce <u>2 types of toxins</u></p> <ol style="list-style-type: none"> ① Lethal ② haemolytic Toxin <p>in broth containing veal infusion and glucose but Not in ordinary broth.</p>	<p>produce <u>8 Types of toxins</u></p> <p>(α, B, γ, δ, E, θ, Zeta and EXa)</p> <p><u>C. novyi</u>:</p> <ol style="list-style-type: none"> ① <u>Type A</u> → α, γ, δ and E ② <u>Type B</u> → α, B, Zeta and EXa ③ <u>Type C</u> → Non-toxicogenic ④ <u>Type D</u> → B, Theta and EXa
γ (Gamma)	μ (Mu)										
δ (Delta)	η (eta)										
θ (Theta)	κ (Kappa)										
ν (nu)	λ (Lambda)										

④

pathogenicity

C. Tetani

Tetanus (Lockjaw) disease. كزاز الفم

It arises from:

① Contamination of deep wounds or post-operations with spores of the organism.

② post-natal tetanus:

occurs in adults post-abortion or post-delivery.

③ Neonatal tetanus:

occurs in newly born animals as a result of umbilical infection.

④ Self infection:

Where spores are present naturally in the intestinal tract (faeces) of animals.

⑤ Idiopathic tetanus:

site of infection is undiscoverable.

● Tetanospasmin is absorbed through one of the following pathways:

↓
Motor nerve ending to spinal cord

accompanied by spasms beginning from the wounded limb to the head and neck (ascending tetanus)

↓
through lymphatics to blood stream then directly to brain

accompanied by spasms beginning from the head and neck to the limbs (descending tetanus)

C. botulinum

① Types A, B, E, F and G →

Botulism in man and animals

② Type C:

→ Limber neck in fowls
→ ALkali disease in ducks
→ Lamie sickness in cattle

③ Type D:

Lamziekte (Loin disease) in cattle

Botulism:

is an intoxication (Food intoxication) and not a bacterial infection.

→ The organism does not multiply in the living tissue but it multiplies in conserved food or decaying matter.

C. perfringens (C. welchii)			C. Septicum	
Type	diseases	Route of infection	diseases	Route
A	① Malignant oedema (gas gangrene) in man and animals.	Wound	1- Malignant oedema in man and animals	Wound
	② Malignant oedema (gangrenous dermatitis) in chickens	Wound	2- gangrenous dermatitis in chicken	Wound
	③ Enterotoxaemia in sheep and calves	ingestion	3- Braxy (Bradsot) in sheep.	ingestion
	④ Food poisoning in man	ingestion		
B	1- Lamb dysentery 2- enterotoxaemia in calves	Ingestion	C. chauvoei	
C	1- struck disease in adult sheep 2- enterotoxaemia in calves 3- Necrotic enteritis in chickens	ingestion		
D	1- pulpy kidney in young sheep 2- enterotoxaemia in calves	ingestion		
E	1- Necrotic enteritis in man 2- enterotoxaemia	ingestion	diseases	Route
F	Enterotoxaemia	ingestion	1- Symptomatic anthrax in sheep.	Wound
● diseases caused by C. perfringens type B and C are due to consumption of excessive amounts of Food Low in protein and rich in CHO → because it is accompanied by Low production of pancreatic trypsin or other proteases which destroy B-toxin → so, allowing B-toxin to act in the small intestine.			2- Black quarter (Black Leg) in cattle and buffaloes	ingestion
● Sudden death occurs within 24 hrs due to toxaemia.				

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C. novyi				● Malignant oedema (gas-gangrene) <u>In man and animals:</u> → Caused by <ul style="list-style-type: none"> → C. perfringens type A → C. septicum → C. novyi type A → C. Sordelli → In chicken, It is called <u>gangrenous dermatitis</u> and Caused by <ul style="list-style-type: none"> → C. perfringens type A → C. septicum → C. novyi type A → C. sporogenes With or without staphylococci
Type	diseases	Susceptible animals	Route	
A	① Malignant oedema ② gangrenous dermatitis ③ Swelled head in breeding rams	Man and animals chickens Ram	Wound	
B	1- <u>Black disease</u> (Infectious necrotic hepatitis) → Common in animals suffer from Liver Fluke infestation. 2- Swelled head in breeding rams	Sheep and Cattle Ram	ingestion Wound	● ulcerative enteritis (Quail disease): Caused by C. Colinum
C	Non-toxicogenic			
D (C. haemolyticum)	<u>Red Water disease</u> (Infectious ictero-haemoglobinurea or Bacillary haemoglobinurea)	Cattle and Sheep	ingestion	● omphalitis (Mushy chick disease): Caused by clostridia with other bacteria (E. coli)

Immunity

I Natural (Innate) immunity:

- dogs, cats and fowls are naturally resistant to tetanus.
- Man, equines, dogs and fowls are naturally resistant to *C. chauvoei* infection

II Acquired (adaptive) immunity:



1 - active Immunity

by vaccination

a Vaccines used for clostridia:

1 - aluminium phosphate precipitated toxoid: deep I/M

→ used for *C. tetani* and *C. novyi*

2 - alum precipitated formalized whole culture vaccine:

→ It contains inactive organisms and toxoids → for *C. perfringens* type A, *C. septicum* and *C. chauvoei*
 → deep I/M

3 - Formalized whole culture vaccine:

→ *C. perfringens* type B and D and *C. novyi*
 → S/C

4 - polyvalent vaccine for malignant oedema (gas gangrene):

It is prepared from *C. perfringens* type A, *C. septicum* and *C. novyi* type A.

5 - Co-vaccine (collective vaccine for most or all anaerobic diseases):

→ It is prepared from 8 strains
 → given S/C

N.B:

Preparation of tetanus toxoid:

by addition of 0.3% formalin to culture filtrate → incubated for several weeks → then, the toxoid is purified by precipitation with aluminium phosphate.

(b) Vaccination against Lamb dysentery and pulpy Kidney:

(C. perfringens type B and D)

→ as the infection occurs early in Lambs from few hrs of Life (Lamb dysentery) or from 3 months of Life (pulpy Kidney) → induction of immunity depends on natural passive immunity from the mother to the newly born Lambs through Colostrum.

1-Vaccination of pregnant ewe:

2 doses of formalized whole culture of C. perfringens type B and D.

1st dose: 5 wks before parturition (S/C)

2nd dose: 2 wks after 1st dose (the vaccine is active for mother and passive for Lamb)

→ after parturition, the Lamb must receive Colostrum from the immunized dams → because it contains high conc. of B and E antitoxins (natural passive immunity)

2-Vaccination of Lambs:

3 months old Lambs are vaccinated by formalized C. perfringens type B and D vaccine →

3 successive injections with one month interval (active vaccination of Lambs).

2-passive immunity

- used only for C. tetani
- by using antitetanic serum (antitoxin) which gives immediate protection and remain for one month.
- used for

prophylactic measures

2 mL (1500 IU/mL) are injected IM immediately post-accidents or post-operations or in severe burns.

therapeutic measures

Single Large dose of 10-20 mL is injected I/M in human and in highly expensive race horses.

- Repeated dose of anti-tetanic serum may cause serum sickness

3-Simultaneous

(active and passive immunity in the same animal)

- applied by injection of tetanus toxoid on one side of neck and antitetanic serum on the other side.

- It gives immediate protection as well as long lasting immunity at the same time.